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<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  (Use as many sheets as necessary)				<b>Complete if Known</b>	
				Application Number	09/863,765
				Filing Date	May 23, 2001
				First Named Inventor	Z. Wang
				Art Unit	1631
				Examiner Name	Shubo Zhou, Ph.D.
Sheet	1	of	1	Attorney Docket Number	09373/100H812-US2

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	Document Number Number-Kind Code <sup>2</sup> (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
AE	AA	US-6,518,065-B1	02-11-2003	Stemmer	
AE	AB	US-6,365,408-B1	04-02-2002	Stemmer	
AE	AC	US-6,323,030-B1	11-27-2001	Stemmer	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. <sup>1</sup>	Foreign Patent Document Country Code <sup>3</sup> -Number <sup>4</sup> -Kind Code <sup>5</sup> (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T <sup>6</sup>

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NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. <sup>1</sup>	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T <sup>2</sup>
AE	CA	M. Ostweimer et al., "A Combinatorial Approach to Hybrid Enzymes Independent of DNA Homology," <i>Nature Biotechnology</i> , December 1999, vol. 17, pp. 1205-1209.	

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DOCKET NO.: 9373/1H812 US2 SERIAL NO: 09/863,765  
APPLICANT: Zhen-Gang Wang et al. FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

**U.S. PATENT DOCUMENTS**

<u>*EXAMINER INITIALS</u>	<u>DOCUMENT NUMBER</u>	<u>DATE</u>	<u>NAME</u>	<u>CLASS</u>	<u>SUBCLASS</u>	<u>FILING DATE</u>
VA	1. 5,605,793	2/25/97	Stemmer	435	6	2/17/94
VA	2. 5,741,691	4/21/98	Arnold, <i>et al.</i>	435	197	1/23/96
VA	3. 5,811,238	9/22/98	Stemmer, <i>et al.</i>	435	6	11/30/95
VA	4. 5,830,721	11/3/98	Stemmer, <i>et al.</i>	435	172.1	3/4/96

**FOREIGN PATENT DOCUMENTS**

<u>*EXAMINER INITIALS</u>	<u>DOCUMENT NUMBER</u>	<u>DATE</u>	<u>COUNTRY</u>	<u>CLASS</u>	<u>SUBCLASS</u>	<u>TRANSLATION YES NO</u>
VA	5. WO 95/22625	8/24/95	U.S.	C12Q	1/68	
VA	6. WO 97/20078	6/5/97	U.S.	C12Q	1/68	
				C12N	15/00	
				C07K	14/00	
CA	7. WO 98/42832	10/1/98	U.S.	C12N	15/09	
				C12P	19/34	

**OTHER REFERENCES****(INCLUDING AUTHOR, TITLE DATE, PERTINENT PAGES, ETC.)**

\*EXAMINER  
INITIALS

- CA 8. Affholter & Arnold, "Engineering a revolution," *Chemistry in Britain*, 1999, 35:48-51.
- CA 9. Almassy et al., "Structures of apo and complexed *Escherica coli* glycinamide ribonucleotide transformylase", *Proc. Natl. Acad. Sci. U.S.A.*, 1992, 89:6114-6118.
- CA 10. Current Protocols in Molecular Biology, (Ausubel, F.M. et al. (Eds.), John Wiley & Sons, Inc. 1994).  
This is a general textbook of several hundred pages which we can provide upon request.

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NOV 29 2001

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CONFIRMATION NO: 9136

**\*EXAMINER  
INITIALS**

11. *Animal Cell Culture*, (R.I. Freshney, ed., Oxford University Press Inc. 1986). This is a general textbook of several hundred pages which we can provide upon request.
12. Arnold, "Advances in protein chemistry. Preface.", *Adv. Protein Chem.* 2000, **55**:ix-xi
13. Bairoch & Apweiler, "The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000", *Nucl. Acids Res.*, 2000, **28**:45-48.
14. B.E. Perbal, *A Practical Guide to Molecular Cloning* (Wiley, John & Sons Inc., 1984). This is a general textbook of several hundred pages which we can provide upon request.
15. Benkovic et al., "A combinatorial approach to hybrid enzymes independent of DNA homology", *Nature Biotechnology*. 1999, **17**:1205-1209.
16. Berman et al., "The Protein Data Bank", *Nucl. Acids Res.*, 2000, **28**: 235-242.
17. Bogarad & Deem, "A hierarchical approach to protein molecular evolution", *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**:2591-2595.
18. Brooks B.R. et al., "CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations", *J. Comp. Chem.*, 1983, **4**:187-217.
19. Colombo G & Mrez KM, "Stability and Activity of Mesophilic Subtilisin E and its Thermophilic Homolog: Insights from Molecular Dynamics Simulations", *J. Amer. Chem. Soc.*, 1999, **121**: 6895-6903.
20. Cornell et al., "A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules", *J. Amer. Chem. Soc.*, 1995, **117**: 5179-5197.
21. Crameri et al., "DNA shuffling of a family of genes from diverse species accelerates directed evolution", *Nature*, 1998, **391**:288-290.
22. Dahiyat et al., "Automated design of the surface positions of protein helices", *Protein Science*, 1997, **6**:1333-1337.
23. Dahiyat & Mayo, "De Novo Protein Design: Fully Automated Sequence Selection", *Science*, 1997, **278**: 82-87.

*J. P. H. H. H.* 4/14/04

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NOV 29 2001

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(REV. 7-80)

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APPLICANT: Zhen-Gang Wang et al. FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

\*EXAMINER  
INITIALS

24. De Souza, et al., "Intron positions correlate with module boundaries in ancient proteins", *Proc. Natl. Acad. Sci. USA*, 1996, **93**: 14632-14636.
25. DNA Cloning: A Practical Approach, Volumes I and II (B.D. Hames & D.M. Glover eds., Oxford University Press Inc., 1985). This is a general multiple volume textbook of several hundred pages which we can provide upon request.
26. Dube et al., "Selection of new biologically active molecules from random nucleotide sequences", *Gene*, 1993, **137**:41-47.
27. Eisenberg, D & McLachlan AD, "Solvation Energy in Protein Folding and Binding", *Nature*, 1986, **319**: 199-203.
28. Eisenberg D & Wesson L, "Atomic solvation parameters applied to molecular dynamics of proteins in solution", *Protein Science*, 1992, **1**: 227-235.
29. Gilbert, et al., "Origin of Genes", *Proc. Natl. Acad. Sci. USA*, 1997, **94**: 7698-7703.
30. Go, M., "Correlation of DNA exonic regions with protein structural units in haemoglobin", 1981, *Nature*, **291**: 90-92.
31. Go, M., "Modular structural units, exons, and function in chicken lysozyme", *Proc. Natl. Acad. Sci. USA*, 1983, **80**: 1964-1968.
32. Gordon and Mayo, "Radical Performance Enhancements for Combinatorial Optimization Algorithms based on the Dead-End Elimination Theorem", *J. Comp. Chem.*, 1998, **19**(13): 1505-1514.
33. Hendsch ZS, Tidor B, "Do salt bridges stabilize proteins? A continuum electrostatic analysis", *Protein Science*, 1994, **3**: 211-226.
34. Hennecke, et al., "Random Circular Permutation of DsbA Reveals Segments that are Essential for Protein Folding and Stability", 1999, *J. Mol. Biol.*, **286**: 1197-1215.
35. Hogue et al., "Structure Databases", *Methods Biochem. Anal.*, 1998, **39**: 46-73.
36. John H. Holland, Adaptation in Natural and Artificial Systems, (MIT Press, 1992) This is a general textbook of several hundred pages which we can provide upon request.

*ShuBren* 4/14/04

RECEIVED

NOV 29 2001

TECH CENTER 1000 OF 8  
NOV 29 2001 (REV. 7-80)

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CONFIRMATION NO: 9136

\*EXAMINER  
INITIALS

*AG*

37. Immobilized Cells and Enzymes: A Practical Approach (IRL Press, 1986). This is a general textbook which we can provide upon request.
38. Jackson SE, et al., "Effect of Cavity-Creating Mutations in the Hydrophobic Core of Chymotrypsin Inhibitor 2", *Biochemistry*, 1993, **32**: 11259-11269.
39. Jermutus, et al., "Structure-based chimeric enzymes as an alternative to directed enzyme evolution: phytase as a test case", *J. Biotech.*, 2001, **85**: 15-24.
40. Joo et al., "Laboratory evolution of peroxide-mediated cytochrome P450 hydroxylation", *Nature*, 1999, **399**: 670-673.
41. Kolkman & Stemmer, "Directed evolution of proteins by exon shuffling", *Nature Biotechnology*, 2001, **19**: 423-428.
42. Lazar GA, Desjarlais JR & Handel TM, "De Novo Design of the Hydrophobic core of ubiquitin", *Protein Science*, 1997, **6**:1167-1178.
43. Lee C & Levitt M, "Accurate prediction of the stability and activity effects of site-directed mutagenesis on a protein core", *Nature*, 1991, **352**: 448-451.
44. Lobkovsky et al., "Evolution of an enzyme activity: Crystallographic structure at 2-Å resolution of cephalosporinase from *ampC* gene of *Enterobacter cloacae* P99 and comparison with a class A penicillinase", *Proc. Natl. Acad. Sci. U.S.A.*, 1993, **90**:11257-11261.
45. MacKerell et al., in *The Encyclopedia of Computational Chemistry*, Vol. 1:271-277, John Wiley & Sons, Chichester, 1998.
46. Malakaukas & Mayo, "Design, structure and stability of a hyperthermophilic protein variant", *Nature Structural Biology*, 1998, **5**:470-475.
47. Marchler-Bauer et al., "MMDB: Entrez's 3D structure database", *Nucl. Acids Res.* 1999, **27**:240-243.
- AG* 48. Mayo SL, Olafson BD & Goddard WAG, "DREIDING: A Generic Force Field for Molecular Simulations", *J. Phys. Chem.*, 1990, **94**: 8897-8909.

*[Signature]* 4/14/04

**LIST OF REFERENCES CITED BY APPLICANT**

(Use Several Sheets if Necessary)

DOCKET NO.: 9373/1H812 US2      SERIAL NO: 09/863,765  
APPLICANT: Zhen-Gang Wang et al.      FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

**\*EXAMINER  
INITIALS**

49. Mitra et al., "A Novel Structural Basis for Membrane Association of Protein: Construction of a Chimeric Soluble Mutant of (S)-Mandelate Dehydrogenase from *Pseudomonas putida*", *Biochemistry*, 1993, **32**: 12959-12967.

50. Miyazaki et al., "Directed Evolution Study of Temperature Adaptation in Psychrophilic Enzyme", *J. Mol. Biol.* 2000, **297**:1015-1026.

51. Miyazaki & Arnold, "Exploring Nonnatural Evolutionary Pathways by Saturation Mutagenesis: Rapid Improvement of Protein Function", *J. Molecular Evolution*, (1999) **49**:716-720.

52. Moore & Arnold, "Directed Evolution of a *para*-nitrobenzyl esterase for aqueous-organic solvents", *Nature Biotechnology*, 1996, **14**: 458-467.

53. Ness et al., "Molecular Breeding - the natural approach to enzyme design", *Advances in Protein Chemistry*, 2000, **55**: 261-292.

54. Ness et al., "DNA shuffling of subgenomic sequences of subtilisin", *Nature Biotechnology*, 1999, **17**:893-896.

55. Nielsen JE, Andersen KV, Honig B, Hooft RWW, Klebe G, Vriend G, & Wade RC, "Improving macromolecular electrostatics calculations", *Protein Engineering*, 1999, **12**: 657-662.

56. Nikolova et al., "Semirational design of active tumor suppressor p53 DNA binding domain with enhanced stability", *Proc. Natl. Acad. Sci. U.S.A.*, 1998, **95**:14675-14680.

57. NMR of Macromolecules: A Practical Approach, (G.C.K. Roberts, Ed., Oxford University Press Inc., New York, 1993) This is a general text book of several hundred pages which we can provide upon request.

58. Nucleic Acid Hybridization, (B.D. Hames & S.J. Higgins, eds., Oxford University Press Inc., 1985). This is a general text book of several hundred pages which we can provide upon request.

59. Oligonucleotide Synthesis (M.J. Gait ed., Oxford University Press Inc., 1984). This is a general text book of several hundred pages which we can provide upon request.

60. Ostermeier et al., "Incremental truncation as a Strategy in the engineering of novel biocatalysts", *Bioorganic & Medicinal Chem.*, 1999, **7**: 2139-2144.

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(REV. 7-80)

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APPLICANT: Zhen-Gang Wang et al. FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

\*EXAMINER  
INITIALS

61. Pabo CO & Suchanek EG, "Computer-Aided Model-Building Strategies for Protein Design", *Biochemistry*, 1986, 25: 5987-5991.
62. Pachenko, et al., "Foldons, protein structural modules, and exons", *Proc. Natl. Acad. Sci. USA*, 1996, 93: 2008-2013.
63. Pikuleva, et al., "Studies of Distant Members of the P450 Superfamily (P450scs and P450c27) by Random Chimeragenesis", *Archives of Biochem. and Biophys.*, 1996, 334:183-192.
64. Reeck et al., "'Homology in Proteins and Nucleic Acids: A Terminology Muddle and a Way out of it", *Cell* 1987, 50:667.
65. Rossman, M. G. & Liljas, A., "Recognition of Structural Domains in Globular Proteins", *J. Molec. Biol.*, 1974, 85: 177-181.
66. Saiki et al., "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase", *Science*, 1988, 239:487-491.
67. Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 1989). This is a three volume general practitioners book of several hundred pages which we can provide upon request.
68. Schmidt-Dannert, et al., "Molecular breeding of carotenoid biosynthetic pathways", *Nature Biotechnology*, 2000, 18:750-753.
69. Schneider JP, Lear JD, DeGrado WF, "A Designed Buried Salt Bridge in a Heterodimeric Coiled Coil," *J. Am. Chem. Soc.*, 1997, 119:5742-5743.
70. Shimoji et al., "Design of a Novel P450: A Functional Bacterial - Human Cytochrome P450 Chimera", *Biochemistry*, 1998, 37: 8848-8852.
71. Sidelar CV, Hendsch ZS, Tidor B, "Effects of salt bridges on protein structure and design", *Protein Science*, 1998, 7: 1898-1914.
72. Sieber et al., "Libraries of hybrid proteins from distantly related sequences", *Nature Biotechnology*, 2000, 19, 456-460.

*Shubor* 4/15/04

**LIST OF REFERENCES CITED BY APPLICANT**

(Use Several Sheets if Necessary)

DOCKET NO.: 9373/1H812 US2 SERIAL NO: 09/863,765  
APPLICANT: Zhen-Gang Wang et al. FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

\*EXAMINER  
INITIALS

73. Skandalis et al., "Creating novel enzymes by applied molecular evolution", *Chem. Biol.*, 1997, 4:889-898.

74. Stemmer, "DNA shuffling by random fragmentation and reassembly: *In vitro* recombination for molecular evolution", *Proc. Natl. Acad. Sci.*, 1994, 91 :10747-10751.

75. Stemmer, "Rapid evolution of a protein *in vitro* by DNA shuffling", *Nature*, 1994, 370:389-391.

76. Stikoff D, Lockhart DJ, Sharp KA & Honig B, "Calculation of electrostatic effects at the amino-terminus of an alpha-helix", *Biophys. J.*, 1994, 67: 2251-2260.

77. Street AG & Mayo SL, "Pairwise calculation of protein solvent-accessible surface areas", 1998, 3: 253-258.

78. Street & Mayo, "Computational protein design", *Structure*, 1999, 7: R105-R109.

79. Tatusova, T. A. & Madden T. L., "BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.*, 1999, 174:247-250.

80. Tsai, C-J., et al., "Anatomy of protein structure: Visualizing how a one-dimensional protein folds into a three-dimensional shape", *Proc. Nat. Acad. Sci., USA*, 2000, 97:12038-12043.

81. Volkov et al., "Methods for *in Vitro* DNA Recombination and Random Chimeragenesis", *Methods Enzymol*, 2000, 328: 447-456.

82. Volkov et al., "Recombination and chimeragenesis by *in vitro* heteroduplex formation and *in vivo* repair", *Nucl. Acids Res.*, 1999, 27:e18, i-vi.

83. Wang et al., "MMDB: 3D structure data in Entrez", *Nucl. Acids Res.*, 2000, 28:243-245.

84. Weiner et al., "A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins" *J. Amer. Chem. Soc.*, 1984, 106:765-784.

85. Weiner et al., "An All Atom Force Field for Simulations of Proteins and Nucleic Acids", *J. Comp. Chem.*, 1986, 7:230-252.

*Handwritten signature* 4/15/04



**LIST OF REFERENCES CITED BY APPLICANT**

(Use Several Sheets if Necessary)

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APPLICANT: Zhen-Gang Wang et al. FILING DATE: 5-23-01  
CONFIRMATION NO: 9136

\*EXAMINER  
INITIALS

86. Woods et al., "Molecular Mechanical and Molecular Dynamical Simulations of Glycoproteins and Oligosaccharides. 1. GLYCAM\_93 Parameter Development", *J. Phys. Chem.*, 1995, **99**:3832-3846.
87. Zhao & Arnold, "Combinatorial protein design: strategies for screening protein libraries", *Curr. Op. St. Biol.*, 1997, **7**: 480-485.
88. Zhao & Arnold, "Directed evolution converts subtilisin E into a functional equivalent of thermitase", *Protein Engineering*, 1999, **12**:47-53.
89. Zhao & Arnold, "Optimization of DNA shuffling for high fidelity recombination", *Nucleic Acids Res.*, 1997, **25**:1307-1308.
90. Zhao et al., "Molecular evolution by staggered extension process (StEP) in vitro recombination," *Nat. Biotechnology*, 1998, **16**(3):258-61.

 4/15/04